

**WHAT IS CLAIMED IS:**

- 1        1.        A method comprising:  
2                freezing a biochemically active tissue sample, wherein freezing includes:  
3                        immersing the tissue sample in cooling fluid;  
4                        circulating the cooling fluid past the tissue sample at a substantially  
5                                constant predetermined velocity and temperature to freeze the tissue  
6                                sample such that the tissue sample is vitrified; and wherein  
7                                the tissue sample maintains its anatomical structure and remains  
8                                biochemically active after thaw;  
9                thawing the tissue sample; and  
10               examining the thawed tissue sample.
  
- 1        2.        The method as in Claim 1, further comprising sectioning the tissue sample.
  
- 1        3.        The method as in Claim 1, wherein examining the thawed tissue sample  
2               includes histological examination.
  
- 1        4.        The method as in Claim 1, wherein examining the thawed tissue sample  
2               includes ultrastructural examination.
  
- 1        5.        The method as in Claim 1, wherein examining includes the use of  
2               immunohistochemistry examination.
  
- 1        6.        The method as in Claim 5, wherein immunohistochemistry includes  
2               fluorescent labeled antibody staining.

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- 1        7.        The method as in Claim 1, wherein more than about 55 percent of the tissue  
2                   sample exhibits no damage to cellular anatomical structure and remains  
3                   biochemically active after thaw.
  
- 1        8.        The method as in Claim 1, wherein more than about 45 percent of the tissue  
2                   sample exhibits no damage to cellular anatomical structure and remains  
3                   biochemically active after thaw.
  
- 1        9.        The method as in Claim 1, wherein more than about 85 percent of the tissue  
2                   sample maintains its anatomical structure and remains undamaged after thaw.
  
- 1        10.       The method as in Claim 1, wherein the cooling fluid is maintained at a  
2                   temperature of between about -20 degrees centigrade and about -30 degrees  
3                   centigrade.
  
- 1        11.       The method as in Claim 1, wherein the velocity of the cooling fluid past the  
2                   tissue sample is about 35 liters per minute per foot of cooling fluid through an  
3                   area not greater than about 24 inches wide and 48 inches deep.
  
- 1        12.       The method as in Claim 1, wherein, the cooling fluid is circulated by a  
2                   motor/impeller assembly immersed in the cooling fluid.
  
- 1        13.       The method as in Claim 1, further comprising circulating the cooling fluid past  
2                   a multi-path heat exchanging coil submersed in the cooling fluid, and wherein  
3                   the heat exchanging coil is capable of removing at least the same amount of  
4                   heat from the cooling fluid, as the cooling fluid removes from the tissue  
5                   sample.

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- 1        14.    A method for use in preparing a tissue sample for examination, the method  
2            comprising:  
3            immersing a biologically active tissue sample in cooling fluid; and  
4            freezing the tissue sample directly to a temperature higher than about -30  
5                degrees centigrade by circulating the cooling fluid past the tissue sample at  
6                a substantially constant predetermined velocity and temperature such that  
7                the tissue sample is vitrified, the tissue sample maintains its anatomical  
8                structure, and the tissue sample remains biochemically active after thaw.
- 1        15.    The method as in Claim 14, further comprising sectioning the tissue sample.
- 1        16.    The method as in Claim 14, further comprising thawing the tissue sample.
- 1        17.    The method as in Claim 14, wherein examination includes histological  
2            examination.
- 1        18.    The method as in Claim 14, wherein examination includes ultrastructural  
2            examination.
- 1        19.    The method as in Claim 14, wherein examination includes the use of  
2            immunohistochemistry examination.
- 1        20.    The method as in Claim 19, wherein immunohistochemistry includes  
2            fluorescent labeled antibody staining.
- 1        21.    The method as in Claim 14, wherein more than about 40 percent of the tissue  
2            sample maintains its anatomical structure and remains biochemically active  
3            after thaw.

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- 1        28.     A system for use in preparing a tissue sample for examination, the system  
2        comprising:  
3                a cooling fluid reservoir configured to receive a biochemically active tissue  
4                sample for immersion in cooling fluid;  
5                one or more cooling fluid circulators configured to circulate said cooling fluid;  
6                a heat exchanging coil for removing heat from said cooling fluid;  
7                a refrigeration unit configured to remove heat from said heat exchanging coil;  
8                and wherein  
9                said cooling fluid reservoir, said one or more circulators, and said refrigeration  
10                unit cooperate to freeze the tissue sample directly to a temperature higher  
11                than about -30 degrees centigrade by circulating the cooling fluid past the  
12                tissue sample at a substantially constant predetermined velocity and  
13                temperature such that the tissue sample is vitrified, the tissue sample  
14                maintains its anatomical structure, and the tissue sample remains  
15                biochemically active after thaw.
- 1        29.     The system as in Claim 28, wherein examination includes histological  
2                examination.
- 1        30.     The system as in Claim 28, wherein examination includes ultrastructural  
2                examination.
- 1        31.     The system as in Claim 28, wherein examination includes the use of  
2                immunohistochemistry examination.
- 1        32.     The system as in Claim 31, wherein immunohistochemistry includes  
2                fluorescent labeled antibody staining.
- 1        33.     The system as in Claim 28, wherein more than about 40 percent of the tissue

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2 sample maintains its anatomical structure and remains biochemically active  
3 after thaw.

1 34. The system as in Claim 28, wherein more than about 80 percent of the tissue  
2 sample maintains its anatomical structure and remains biochemically active  
3 after thaw.

1 35. The system as in Claim 28, wherein more than about 85 percent of the tissue  
2 sample maintains its anatomical structure and remains undamaged.

1 36. The system as in Claim 28, wherein the cooling fluid is maintained at a  
2 temperature of between about -20 degrees centigrade and about -30 degrees  
3 centigrade.

1 37. The system as in Claim 28, wherein the velocity of the cooling fluid past the  
2 tissue sample is about 35 liters per minute per foot of cooling fluid through an  
3 area not greater than about 24 inches wide and 48 inches deep.

1 38. The system as in Claim 28, wherein, the circulator is a motor/impeller  
2 assembly immersed in the cooling fluid.

1 39. The system as in Claim 28, wherein the cooling fluid is circulated past a multi-  
2 path heat exchanging coil submersed in the cooling fluid, and wherein the heat  
3 exchanging coil is capable of removing at least the same amount of heat from  
4 the cooling fluid, as the cooling fluid removes from the tissue sample.